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13. ABSTRACT (Maximum 200 Words) The purpose of this study is to investigate the relationship between breast cancer risk, bone mass, and two polymorphic hormone receptor genes-- the estrogen receptor (ER) and vitamin D receptor (VDR) genes. We will also explore a possible functional mechanism to explain this association. We will recruit 200 new breast cancer cases and 200 controls, ages 40-85, with equal numbers of African-Americans and whites. Bone mineral density (BMD) measurements of the forearm will be obtained. We have enrolled 231 cases and 198 controls, with an age range of 39-84. There is an equitable distribution of the two ethnic groups, with 50.3% of the cohort being white and 49.7% African-American. We enrolled more than 400 subjects because some individuals changed their minds about giving blood, some blood samples were not analyzable, and some subjects have no bone density data due to instrument malfunctions. Preliminary results on the genotype data suggest some differences in frequencies of the ER and VDR genotypes between cases and controls. BMD in the proximal radius of the cases is higher than that of controls when adjusted for age, weight, ethnicity, and HRT use (0.790 vs 0.775 g/cm ²), as hypothesized. Multivariate analyses and relative risk models are forthcoming when all data are entered into the database. We have begun identifying cases and devising the experiments for our final objective, which is to investigate the responsivity to estrogen of the polymorphic ER genotypes.				
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INTRODUCTION

The **objective** of this study is to investigate the relationship between two polymorphic genes that are potential determinants of bone mass, and breast cancer risk, in African-American and white women, and to explore a possible functional mechanism to explain this association. Our hypothesis is that variations in these receptor genes affect the responsivity of bone and breast tissue to a given level of steroid exposure, and therefore correspond to variations in bone mass and the risk of breast cancer. That is, there may be genetically-determined individual variation in responsivity to identical stimuli that could explain the reported relationship between a higher bone mass and a higher breast cancer risk¹⁻³. Our **specific aims** and **hypotheses** are as follows:

1. To compare bone mass and the distribution of genotypes of the VDRG and ERG among 200 new breast cancer cases and 200 controls. Half of each sample will be white ethnicity, the other half African-American. Our **hypothesis** is that: The breast cancer cases will have a higher bone mass and a higher prevalence of the genotypes that are associated with high bone mass; the two ethnic groups will also differ but there are insufficient data to predict in what way they will differ.
2. To identify variations within the DNA sequence encoding the structural elements of the ERG; we **hypothesize** that these will correspond with the recognized polymorphic allotypes.
3. Our ultimate aim, which follows logically from Aim 2, is to determine the significance of the variations in estrogen receptor (identified in Aim 2) to the stimulation of an estrogen responsive reporter gene regulated by promoters of diverse complexity. Our **hypothesis** is that the variants associated with elevated responsivity of the cell to estrogen will be more prevalent in the breast cancer cases compared with controls

BODY

Progress report for each task relevant to Year 2:

Approved Statement of Work

General: Recruitment and data collection for Specific Aim 1 will take place over the first 2.5 years of the study. New breast cancer patients will be recruited and then matched controls will be recruited in either a concurrent fashion, if practicable, or in a staggered design in which patients are recruited over several weeks and then matched controls are recruited, and the cycle is repeated. This may be necessary because although the bone densitometer is "portable" it is not practical to move it frequently. Analyses of genotypes will be ongoing and will finish 3 months after recruitment ends. Laboratory analyses for Specific Aim 2 will take place in Year 3 after most of the genotype data are available.

Progress: We enrolled 231 cases and 198 controls. Our final sample size (n=429) is greater than our target of 400 because some subjects were unwilling to provide blood samples or they were not successfully analyzed for genotypes. A second reason is that the pDEXA instrument was out of service for 4-6 weeks over the 2.5 years of the study, and bone density measurements are missing for some subjects. The total number of subjects with pDEXA data as well as data on at least one

of the genetic loci, is 396. There are approximately 20 more blood samples to be analyzed, so that over 400 subjects will have complete data sets.

Year 2: October 1999-September 2000

TECHNICAL OBJECTIVE 3: To recruit an additional 80 white and 80 black new breast cancer patients and controls (40 each within ethnic groups.

Progress: Recruitment was completed in July 2001. Subjects ranged in age from 39 to 84 years, with a mean of 56.3 ± 10.8 years. There is an equitable distribution of the two ethnic groups, with 50.3% of the cohort being white and 49.7% African-American. Descriptive statistics are provided in Table 1.

TABLE 1: DESCRIPTIVE STATISTICS (MEAN \pm S.D.) OF DEMOGRAPHIC DATA FOR CASES AND CONTROLS, BY ETHNIC GROUP

Variables	Breast Cancer Cases		Controls	
	White N=120	African-American N=111	White N=96	African-American N=102
Age (yrs)	57.7 ± 10.9	57.0 ± 11.3	55.0 ± 10.6	55.0 ± 10.2
Height (cm)	163.0 ± 6.6	162.7 ± 7.6	164.3 ± 7.5	163.1 ± 17.6
Weight (kg)*	73.0 ± 16.5	82.9 ± 22.2	70.3 ± 14.5	81.0 ± 22.4
Body Mass Index (kg/m ²)*	27.5 ± 6.3	31.4 ± 8.5	26.1 ± 5.3	30.1 ± 7.2
Age at Menarche (yrs)	12.5 ± 1.4	12.6 ± 1.8	12.5 ± 1.5	12.8 ± 1.6
Age at Menopause (yrs)#	49.7 ± 5.6	47.9 ± 7.2	49.0 ± 4.9	$46.0 \pm 7.0^{**}$

*Significant ethnic differences in cases and controls, $p < 0.001$

**Significant ethnic difference in controls, $p = 0.005$

#Note: 270 of the 428 subjects were postmenopausal

DISCUSSION: The data in Table 1 indicate that the cases and controls are very similar in mean age, height, and age at menarche. There are significant ethnic differences in BMI within both the case

and control groups ($p < .001$). Among the controls, African-Americans had a significantly lower age at menopause ($p = 0.005$). However, there are no significant differences between cases and controls within ethnic group. That is, the African-American cases do not differ significantly from the African-American controls, and likewise for the white cases and controls ($p > 0.05$).

Year 2: October 1999-September 2000

TECHNICAL OBJECTIVE 2: To determine the genotypes for the VDRG and ERG in the breast cancer patients and controls.

Task 4: Months 3-33: Laboratory assistant under Dr. Wooley's direction will perform genetic analyses. Results will be entered into study database.

Progress: Genetic analyses were done in batches and entered at intervals into the database. All but one batch have been analyzed and entered. To date, 400 subjects have data for the PvuII haplotype of the ERG; 398 have data for the XbaI haplotype; and 407 have data for the BsmI haplotype of the VDG. On average, 94% of the subjects have genotype data already in the database. Summaries of the genotype data that are presently available are provided in the tables below.

TABLE 2A: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES (PVUII AND XBAI) IN BREAST CANCER CASES (N=213)

PVUII HAPLOTYPE	XBAI HAPLOTYPE		
	XX	Xx	xx
PP	24(11.3%)	18(8.5%)	11 (5.2%)
Pp	3 (1.4%)	66 (31.0%)	38 (17.8%)
pp	1 (0.5%)	3 (1.4%)	49 (23%)

TABLE 2B: FREQUENCIES (ACTUAL AND PERCENT) OF ESTROGEN RECEPTOR GENE HAPLOTYPES (PVUII AND XBAI) IN CONTROLS (N=184)

PVUII HAPLOTYPE	XBAI HAPLOTYPE		
	XX	Xx	xx
PP	16 (8.7%)	14 (7.6%)	17 (9.2%)
Pp	0	65 (35.3%)	32 (17.4%)
Pp	0	3(1.6%)	37(20.1%)

DISCUSSION: The genotype data in Tables 2a and 2b suggest some differences in frequencies of the estrogen receptor gene between cases and controls. No definitive statement can be made until all the genetic data are in the database and a statistical analysis is performed. These data do not appear to support the model we developed from our pilot data: that there are four genotypes of

the ERG (XXPp, XXpp, xxPP, Xxpp) found in low frequency in cases compared with controls. At this time, these do not appear to differentiate the two groups, but a different combination may be identified during statistical analyses. The VDR genotype frequencies are shown in Table 3.

TABLE 3: FREQUENCIES (ACTUAL AND PERCENT) OF VITAMIN D RECEPTOR GENE BSM1 HAPLOTYPES IN CASES (N=217) AND CONTROLS (N=190)

GROUP	BSM1 HAPLOTYPES		
	BB	Bb	bb
CASES	35 (16.1%)	88(40.6%)	94(43.3%)
CONTROLS	40(21.1%)	66(34.7%)	84(44.2%)

DISCUSSION: The “BB” genotype is found in lower frequency in the cases than controls, while the “Bb” genotype is found in higher frequency in the cases than controls. Based on data in the literature, the “BB” genotype is associated with a lower bone mass than the “bb”, with Bb somewhat intermediate.⁴ Thus, the lower frequency of the “low bone mass” genotype (BB) in the cases is consistent with our hypotheses about genetic determinants of bone mass and their relationship to breast cancer risk. That is, the higher bone mass found in cases may have a genetic underpinning related to variations in the Vitamin D Receptor.

TECHNICAL OBJECTIVE 3: To recruit an additional 80 white and 80 black breast cancer patients and controls (40 of each within each ethnic group).

Task 5: Months 13-24: Study coordinator will attend breast cancer clinics and/or general medicine clinics at least 3 days per week to recruit 2 subjects per day. Blood sample and bone densitometry will be obtained. Specimens will be transported to Dr. Wooley’s lab and stored at -70 degrees. Bone density and questionnaire variables will be entered into the database.

Progress: The study coordinator attended the breast cancer clinic 5 days per week and was successful in recruiting over 429 subjects. Blood samples were transported to Dr. Wooley’s lab for analysis, and other data were entered into the study database. The database is nearly complete, pending the last batch of genetic analyses.

Year 3: October 2000-March 2001

TECHNICAL OBJECTIVE 4: To finish recruitment of subjects (40 white and 40 black) for a total of 400.

Task 6: Months 25-30: Study coordinator will attend breast cancer clinics and/or general medicine clinics at least 3 days per week to recruit 2 subjects per day. Blood sample and bone densitometry will be obtained. Specimens will be

transported to Dr. Wooley's lab and stored at -70 degrees. Bone density and questionnaire variables will be entered into database.

Progress: Same as for Task 5—recruitment has been completed and the final sample size (n=429) is larger than the target of 400. All work has been completed except for one batch of blood samples (approximately 20 specimens). Table 4 shows a preliminary analysis of the bone density data in the postmenopausal women (in whom years post-menopause could be used as a covariate in the model):

TABLE 4: COMPARISON OF ADJUSTED MEANS (\pm STANDARD ERROR) FOR BONE DENSITY IN THE CASES VERSUS CONTROLS

Variables	Breast Cancer Cases N=178	Controls N=161
Adjusted* Distal Forearm BMD (g/cm ²)	0.331 \pm 0.004	0.329 \pm 0.004
Adjusted* Proximal Forearm BMD (g/cm ²)	0.790 \pm 0.007	0.775 \pm 0.007

* Adjusted for age, weight, ethnicity, and use of HRT (ever use); N=339

DISCUSSION: The results for the distal forearm do not show differences between cases and controls. However, the cases have a higher bone density in the radial shaft, compared with controls. Our planned comprehensive statistical analyses of the data will provide an optimal set of covariates for the comparison of bone density in the two groups, but there does appear to be a trend for a higher bone mass in the cases, as expected.

TECHNICAL OBJECTIVE 5: To identify exon(s) on ERG that code for structural protein and which are polymorphic, for Specific Aim 2.

Task 7: Months 25-36: Dr. Wooley will develop probes and primers for sequencing polymorphic variants, in collaboration with Dr. Brooks.

Progress: Work began on this task several months ago. It was decided that tissue specimens rather than blood samples were needed for these experiments. We have obtained IRB approval to ask for subjects' consent to use their tissue (stored in Pathology for clinical purposes). We have identified several dozen cases who have the homozygous genotypes for XbaI and PvuII. To date, we have called 4 subjects, all of whom gave consent. We await a response from Pathology about the availability of tissue.

TECHNICAL OBJECTIVE 6: To test hypotheses and report study results.

Task 8: Months 31-36. Statistical analyses of data, with interpretation by panel of co-investigators; final report and manuscript preparation.

Progress: Dr. Richard Severson, the cancer epidemiologist participating in this project, has been given the database and is exploring the data. He and the P.I. will work together on the planned analyses. It is expected that work will be completed over the next 3 months. (We were granted a no-cost extension on this project through January 31, 2002).

KEY RESEARCH ACCOMPLISHMENTS

- Recruitment of 231 newly diagnosed breast cancer cases and 198 controls.
- Recruitment of nearly equal numbers of both African-American and white cases and controls.
- Recruitment of subjects over most of the targeted age range.
- The genotyping of all blood samples except one final batch.
- A database that is clean and up to date.

REPORTABLE OUTCOMES

- Development of serum repository for genotyping.
- Database with 231 breast cancer cases and 198 controls
 - Equitable distribution of ethnic groups
 - Comprehensive questionnaire data on medical and reproductive history
- A poster presentation at the ERA OF HOPE Breast Cancer Research Program Meeting, Atlanta, GA, June 2000.⁵
 - A-6: "Bone Mass and Estrogen Receptor Gene in Breast Cancer Cases and Controls." Nelson DA, Darga LL.

CONCLUSIONS

We have amassed a relatively large data set, using a prospective study design, of nearly equal numbers of African-American and white breast cancer cases and controls. These data should prove to be valuable for hypothesis generation and testing beyond the objectives of the current project. Data from the 429 subjects suggest apparent differences between cases and controls in the genotype frequencies of the Estrogen Receptor and Vitamin D Receptor genes.

There is an apparent trend for the "BB" genotype, generally associated with lower bone mass⁴, to be found in higher frequency in the controls than the cases. There is also an apparent trend for a higher radial bone density in the cases compared with the controls. These preliminary observations lend support to our hypotheses. In the next few months, we will work on the laboratory experiments focusing on variants in the ERG and how they relate to estrogen responsiveness.

The knowledge to be gained from this study may provide new tools for assessing breast cancer risk early in life (such as bone mass measurement or genotyping) that could lead to modifications in hormone replacement therapy in postmenopausal women and/or increased surveillance for breast cancer in women with high bone mass.

REFERENCES:

1. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR, for the Study of Osteoporotic Fractures Research Group. Bone mineral density and risk of breast cancer in older women: the Study of Osteoporotic Fractures. *JAMA* 1996;276:1404-1408.
2. Zhang Y, Kiel DP, Kreger BE, Cupples LA, Ellison RC, Dorgan JF, Schatzin A, Levy D, Felson DT. Bone mass and the risk of breast cancer among postmenopausal women. *New Engl J Med* 1997;336:611-617.
3. Zmuda JM, Cauley JA, Ljung BM, Bauer DC, Cummings SR, Kuller LH; The Study of Osteoporotic Fractures Research Group. Bone mass and breast cancer risk in older women: differences by stage at diagnosis. *J Natl Cancer Inst* 2001;93:930-6.
4. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Letters to *Nature* 1994;207:284-287.
5. Nelson DA, Darga LL. Bone mass and estrogen receptor gene in breast cancer cases and controls. DoD Breast Cancer Research Program, Era of Hope Meeting, June 2000, Atlanta, GA. (poster).